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Serological detection of infection dynamics for respiratory viruses among dairy calves

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ABSTRACT

The aim of this study is to reveal infection dynamics of bovine respiratory syncytial virus (BRSV), bovine parainfluenza virus type 3 (PI-3), bovine herpesvirus 1 (BHV-1), bovine viral diarrhea virus (BVDV), bovine adenovirus type 3 (BAV-3) and bovine coronavirus (BCoV), which are important viral pathogens of respiratory disease complex in ruminants. Through such an analysis, the regression period of maternally derived antibodies and optimum vaccination time in calves can be recommended. A total of 10 farms were grouped as large (4)-, medium (2)- and small (4)- sized enterprises according to their animal population. Newborn calves (*n*: 94) delivered during a calendar month on the farms were studied. Blood samples were collected from these calves during their 1st, 2nd, 3rd, 4th, 6th, 8th, 10th and 12th months of age. Blood samples were also taken from their dams during the first sampling. Neutralizing antibody titers were detected using the serum neutralization test (SN_{50}). New PI-3 and BVDV infections at the early stages of life were determined in the calves. Maternal antibodies began to decrease in the 2nd month for BRSV, BHV-1 and BAV-3 (97.8%, 25.5% and 91.4%) and in the 3rd month for PI-3, BVDV and BCoV (85.1%, 67% and 93.6%). It was concluded that maternal antibodies begin to decrease after the 1st month and that the possible first exposure of calves to respiratory viruses is after the 2nd month. Therefore, it is recommended that the first vaccination program including prime and booster doses can be applied between 2 and 4 months of age. Furthermore, re-vaccination of animals at 6 months after the booster dose is also suggested.

1. Introduction

Bovine respiratory disease (BRD) is a common health problem in cattle herds (Gulliksen et al., 2009b), and BRD can be classified as the most important welfare health problem in calves. Financial losses related to BRD are due to animal mortality, body weight loss, yield loss, veterinary fees and medical cost. Although respiratory disease can be found in cattle of all ages, during the decline of passive immunity, weaning time and entrance into the feedlot, calves are most susceptible due to the intensive stress and exposure to pathogens (Muggli-Cockett et al., 1992). On a Dutch dairy farm where 60% of the heifers younger than 3 months old were infected, €31.2 in economic losses were estimated per heifer due to pneumonia. In the same study, it was shown that the calculated losses for one seasonal outbreak were €27.0 per heifer up to 15 months old (Van Der Fels-Klerx et al., 2001). Furthermore, the estimated annual economic loss due to respiratory tract infections is over $600 million in the USA (Smith, 2000). The contribution of BRD to the total morbidity cases in the USA was estimated to be approximately 75% and was similarly reported to be 50–70% of all feedlot deaths (Edwards, 1996; Galyean et al., 1999; Loneragan et al., 2001). The spread of BRD infections among young populations is extremely rapid: 91% of calves can be infected with BRD within the first 27 days after arrival to a feedlot (Buhman et al., 2000). An insufficient level of maternally derived antibodies (Kimman et al., 1988) and adrenocortical-immunosuppressive factors, i.e., stress, crowded barns or bad shelter conditions, can lead to an increased incidence of infection in calves (Gulliksen et al., 2009b).

One or more viral agents as well as some bacteria have been reported in the etiology of BRD (Härtel et al., 2004). The most important viral agents in BRD cases are bovine respiratory syncytial virus (BRSV), bovine herpesvirus type 1 (BHV-1), bovine parainfluenza virus type 3 (PI-3), bovine viral diarrhea virus (BVDV) and bovine adenovirus serotype 1-2-3 and 7 (BAV-1, BAV-2, BAV-3, BAV-7) (Härtel et al., 2004; Hägglund et al., 2006; Autio et al., 2007; Gulliksen et al., 2009a). Bovine rhinovirus, bovine coronavirus and bovine reovirus serotype 3 have occasionally been

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isolated (Kurogi et al., 1976; Richer et al., 1988; Decaro et al., 2008). In addition to *Mycoplasma bovis, Mannheimia haemolytica, Histophilus somni* and *Pasteurella multocida* can accompany these viral agents, with the prognosis of clinical illness becoming dramatically worse (Härtel et al., 2004; Dabo et al., 2008; Rice et al., 2008).

As it is commonly accepted that the primary pathogens of BRD are viral agents, the immunity gap (i.e., the time lapse between the regression period of maternally antibodies and the formation of antibodies by vaccination or between vaccination and clinical protection) becomes even more important in BRD. BVD viruses can persist in the cattle population through persistently infected individuals (IPI) (Mcclurkin et al., 1984), and BHV-1 may create lifelong latency after primary infection (Ackermann et al., 1982); it was also reported that BRSV and BCoV may persist within herds (Heckert et al., 1991; Valarcher et al., 2001). Virus clearance between outbreaks (Alenius et al., 1991; Elvander, 1996) and the re-introduction of new viral strains (Larsen et al., 2000) has been reported, and the seasonal incidence of BRD cases is generally higher during autumn and winter (Stott et al., 1980).

The aim of this study is to reveal the infection dynamics of the most important viral agents involved in BRD and to determine the regression period of maternally derived antibodies and the optimum age for the first vaccination.

2. Materials and methods

2.1. Study area and farm visits

This study was carried out in three locations (Karacabey, Mustafakemalpaşa and Yenişehir) in the Bursa province of Turkey. Blood samples were collected from a total of 10 cattle herds. Depending on the animal density (total number of animals including calves, cows and heifers), the herds were classified as small-scale enterprises (total animal number <20), medium-scale enterprises (total animal number is between 20 and 100) and large-scale enterprises (total animal number >100) (Table 1). The farms were visited monthly accompanied by the farm's veterinarians. Blood samples were collected during the visits. The records for clinical cases in the herd between visits were supplied by the veterinarian, and these clinical cases were also sampled for laboratory diagnosis (data not shown).

2.2. Sampled animals, blood samples and records

All the animals studied at the 10 dairy cattle farms were the Holstein-Fresian breed and were either semi-intensively or closely managed (Table 1). Calves that were born in a one-month period (July 2010) on these farms were selected for this study and then followed for 12 months. Blood samples were collected for serological testing from 94 calves at their 1st, 2nd, 3rd, 4th, 6th, 8th, 10th and 12th months of age between June 2010 and July 2011. None of the calves were vaccinated against the examined viruses during the study.

Dams (*n*: 94) were also sampled at the first sampling period (June 2010) for the demonstration of immunologic status. The dams at the large- and medium-scale enterprises had been vaccinated with an inactivated complete virion vaccine against some of the examined viruses (Table 2). Vaccination programs applied for dams in the farms included first vaccination at 4th month of age and the booster dose at 6th month of age followed by rappel vaccination one year apart. Inactivated BCoV vaccine is applied to the dams 2 months before they give birth.

Blood samples were collected into vacutainer tubes by venipuncture. Serum was separated by centrifugation at 3000 rpm, +4 °C, for 10 min, inactivated in a water bath at 56 °C for 30 min and stored at −20 °C until testing.

During the monthly farm visits, data about the clinical status of the studied calves as well as other calves on the farms were also obtained from the farm veterinarians (Table 3) to follow the circulation of viruses in the herds.

2.3. Viruses and cell cultures

The sampled animals were tested for immunological status against BVDV, BHV-1, PI-3, BRSV, BAV-3 and BCoV. BHV-1 strain Cooper, PI-3 strain SF-4 and BAV serotype 3 were formerly obtained from Department of Virology at Ankara University Faculty of Veterinary Medicine, Turkey. BCoV strain Mebus was obtained from Pendik Veterinary Control and Research Institute, Istanbul, Turkey. BVDV strain NADL and Atue strain of BRSV were obtained from Institute for Virology at Justus-Liebig University Faculty of Veterinary Medicine, Giessen-Germany. The Madin-Derby bovine kidney (MDBK) cell line was used for virus propagation and serum neutralization tests. Dulbecco's MEM supplemented with 10% fetal calf serum (FCS) was used for the cell cultures. Furthermore, the cell line and FCS were tested for the absence of pestivirus contamination throughout the study.

2.4. Detection of viral antibodies

In this study, a serum neutralization test (SN_{50}) was performed for the detection of viral antibodies, as described (Frey and Liess, 1971). A pre-dilution of serum samples, which is also accepted as the minimum positive titer values, at 1:2 for BHV-1 and BRSV, 1:5 for BVDV, PI-3 and BCoV, and 1:16 for BAV-3 were used.

For each serum sample, 2 parallel columns and 6 rows in 96-well microplates were used. To the first rows, 50 μl of pre-diluted sample was added, and twofold dilutions were prepared (1:2–1:64 for BRSV and BHV-1; 1:5–1:160 for PI-3, BVDV and BCoV; 1:16–1:512 for BAV-3). Then, an equal volume of 100$TCID_{50}$ diluted test virus was added. Two wells were used as virus controls (100 μl of the 100$TCID_{50}$ diluted virus) and other two as blanks (100 μl of DMEM). For BHV-1, two hours of incubation period

Table 1
Enterprises used for sampling and their management properties.

Enterprise no.	Region	Enterprise size	Number of animal in the farm	Enterprise type
Enterprise 1	Karacabey	Large	500–1000	Semi-intensive
Enterprise 2	Karacabey	Large	>1000	Semi-intensive
Enterprise 3	Karacabey	Large	>1000	Semi-intensive
Enterprise 4	Yenişehir	Large	500–1000	Semi-intensive
Enterprise 5	Mustafakemalpaşa	Medium	20–100	Semi-intensive
Enterprise 6	Mustafakemalpaşa	Medium	20–100	Semi-intensive
Enterprise 7	Mustafakemalpaşa	Small	<20	Semi-intensive
Enterprise 8	Mustafakemalpaşa	Small	<20	Close barn
Enterprise 9	Mustafakemalpaşa	Small	<20	Close barn
Enterprise 10	Mustafakemalpaşa	Small	<20	Close barn

Table 2
Sampled animal data.

Enterprise no	Enterprise size	Calves data	Cows data	
		Total	Total	Previous vaccinations[a]
1	Large	14	14	BVDV, BHV-1, BCoV
2	Large	31	31	BVDV, BHV-1, BCoV
3	Large	21	21	BVDV type-1, BVDV type-2, BHV-1, BRSV, PI-3 and BCoV
4	Large	6	6	BVDV type-1,BVDV type-2, BHV-1, BRSV, PI-3 and BCoV
5	Medium	9	9	BCoV
6	Medium	3	3	BCoV
7	Small	1	1	–
8	Small	5	5	–
9	Small	2	2	–
10	Small	2	2	–
Total		94	94	

[a] Only the infections subjected in the present study are included.

Table 3
Seasonal distribution of clinically sick animals among the entire calf population at the studied farms.

Sampling period	Season	Clinically sick animals*	%
1st–2nd–3rd months	Summer	22 ($p < 0.001$)**	27.50
4th–6th months	Autumn	29 ($p < 0.001$)	36.25
8th month	Winter	28 ($p < 0.001$)	35.00
10th–12th months	Spring	1	1.25
Total		80	100

*Calves having cough, nasal and eye discharge were accepted as clinically sick.
**p values represent comparison between spring and the other seasons.

for neutralization in 5% CO_2 at 37 °C was applied, whereas the other viruses were incubated for one hour. Then, 50 μl of MDBK cell suspension (3×10^5 cells/ml) was added to each well. The test results were scored with an inverted light microscope after 3–7 days of incubation. The complete inhibition of virus propagation in an individual well was accepted as a positive result. The highest serum dilution with a positive result was recorded as the antibody titer for the tested virus. Samples that were antibody positive at the last dilution were re-tested with the extension of the final dilution rates.

2.5. Statistical analysis

In this study, the geometric mean of antibody titers was used for statistical comparisons. Fischer's exact test was employed for the statistical analysis on the effect of enterprise size and seasonal distribution of clinical signs ($p < 0.05$). A Spearman correlation analysis was used for analyzing the relationship between maternally derived antibody levels and the dam's antibody levels.

3. Results

The serological status and antibody titers of the tested animals were detected by a serum neutralization assay. In the population (n: 94) (Tables 4 and 5) over 96% of the calves were found to be seropositive against BRSV, with changing levels of antibody titers during all sampling periods. The number of PI-3 seropositive calves was increased between the 1st and 2nd months and then a decline occurred up to the 6th month; the number of seropositive calves then began to increase again between the 6th and 8th months. The number of calves seropositive for BHV-1 declined up to the 6th month and then exhibited an increase up to the end of the study. For BVDV, an increase in seropositivity was observed between the 1st and 2nd months, followed by a decrease in the seropositive calf number up to the 4th month. The number of BAV-3 seropositive calves started to decrease after the first month, though an increase was detected between months 6 and 8, and the number of BCoV seropositive calves started to decrease from month 2 and increased between months 6 and 10. With the exception of BRSV and BHV-1,

Table 5
Monthly changes in the mean antibody titers of calves (n: 94).

	Calve's data[a]							
	1st m.	2nd m.	3rd m.	4th m.	6th m.	8th m.	10th m.	12th m.
BRSV	515.7	243.1	326.5	362	333.8	432.1	416.4	401.4
PI-3	50.5	45.3	12.6	4.1	4.5	22.4	31.8	34.1
BHV-1	1.7	1.3	1.3	1.1	1.1	1.6	1.5	1.8
BVDV	72.9	89.8	14.6	4.7	15.9	35	43	23.8
BAV-3	66.4	42.9	11.2	7.7	3.2	14.2	25.6	43.4
BCoV	168.5	128.2	35.2	8.7	9.9	23.8	26.2	26.6

[a] For calculating the geometric mean values data from seronegative calves were presented as 1.

Table 4
Percentage of seropositive individuals among calves (n: 94) during the sampling period (%).

	Sampling periods (months of age)							
	1st m.	2nd m.	3rd m.	4th m.	6th m.	8th m.	10th m.	12th m.
BRSV	100 (94/94)	97.8 (92/94)	96.8 (91/94)	100 (94/94)	97.8 (92/94)	96.8 (91/94)	100 (94/94)	100 (94/94)
PI-3	89.3 (84/94)	96.8 (91/94)	85.1 (80/94)	59.5 (56/94)	44.6 (42/94)	75.5 (71/94)	87.2 (82/94)	91.4 (86/94)
BHV-1	35.1 (33/94)	25.5 (24/94)	24.4 (23/94)	13.8 (13/94)	7.4 (7/94)	32.9 (31/94)	21.2 (20/94)	32.9 (31/94)
BVDV	82.9 (78/94)	100 (94/94)	67 (63/94)	56.3 (53/94)	62.7 (59/94)	73.4 (69/94)	73.4 (69/94)	53.1 (50/94)
BAV-3	93.6 (88/94)	91.4 (86/94)	81.9 (77/94)	59.5 (56/94)	57.4 (54/94)	77.6 (73/94)	78.7 (74/94)	90.4 (85/94)
BCoV	96.8 (91/94)	96.8 (91/94)	93.6 (88/94)	74.4 (70/94)	54.2 (51/94)	56.3 (53/94)	63.8 (60/94)	63.8 (60/94)

changes in the antibody titers against the tested viruses were compatible with the number of seropositive calves (Table 5). In addition to the changes in the seropositivity rates an increase was detected in seroconverted (changed from seronegative to seropositive state) calf number mostly at 4th and 10th months of age (Table 6).

High numbers of seropositive animals among the dams were determined for all the viruses, except BHV-1. In the dam population, the seroprevalence of BRSV, PI-3, BHV-1, BVDV, BAV-3 and BCoV was 100%, 93.2%, 35.9%, 96.6%, 96.6% and 93.2%, respectively (Table 7).

Based on the number of seropositive calves; enterprise size, correlation between the dam's antibody status and maternally derived antibodies, and seasonal distributions of clinical signs were examined. There were no significant differences detected among the large-, medium- and small-scale enterprises with regard to BRSV, BAV-3 and BCoV infections ($p > 0.05$). However, a significant difference was determined for PI-3 and BHV-1 between the large- and medium-scale enterprises. There were also significant variations for BVDV between the large- and medium-scale enterprises as well as the large- and small-scale enterprises ($p < 0.005$).

Positive correlations for the transfer of maternally derived antibodies from dam to calf were determined for most of the viruses, except BCoV (BRSV $r = 0.241$, $p = 0.015$; PI-3 $r = 0.483$, $p < 0.001$; BHV-1 $r = 0.574$, $p = 0.001$; BVDV $r = 0.264$, $p = 0.007$; BAV-3 $r = 0.248$, $p = 0.012$; BCoV $r = 0.04132$, $p = 0.752$).

An examination of the seasonal distributions of the clinical signs showed that respiratory viral infections are most common in autumn followed by winter, summer, and then spring ($p < 0.001$) (Table 3).

4. Discussion

The economic impact of BRD is not ignorable in an intensive cattle enterprise. Certain viral pathogens are the main cause of respiratory disease complex in both adult cattle and calves. In the present field study, we analyzed the infection dynamics of BRSV, PI-3, BHV-1, BVDV, BAV-3 and BCoV in intensively managed large-scale ($n > 100$) as well as medium ($20 < n < 100$)- and small ($n < 20$)-scale cattle herds. Although based upon the acquired data, the decline in the maternally derived antibody level was observed, and the possible vaccination time is suggested. More comprehensive studies should be useful to determine the period for vaccination.

Considering the number of seroconverted calves in the population (n: 94), infections by all of the viral agents examined in this study were detected during the sampling period (Table 6). As in the present paper, an association of more than one viral agent in bovine respiratory infections has been shown in many studies (Hägglund et al., 2006; Autio et al., 2007; Gulliksen et al., 2009a).

Consistent with the Hägglund et al. (2006) study in a population, BRSV remained at an almost constant level. The number of animals in different immune statuses due to short-term maternal immunity may enable the continuous circulation of the virus in the herd (Baker et al., 1986). Maternal antibodies against BRSV can be fail to prevent the scattering and replication of the virus (Van Der Poel et al., 1993), and infection can occur in newborns despite a high maternal antibody titer (Baker et al., 1986). Subclinical infection can remain in the herd, and re-infection can occur frequently (Van Der Poel et al., 1993). In addition, following acquired immunity, antibodies against BRSV can be detected years later (Alenius et al., 1991; Elvander, 1996). Thus, the antibodies detected in the follow up period of this study may have derived from previous infections.

In all the sampling periods, at least 96% of the calves in the population were seropositive for BRSV (Table 4). Opposite to Elvander (1996), due to seropositivity the seroconverted calf number is generally low among BRSV-infected calves than PI-3- or BVDV- infected calves during the sampling period (Table 6).

When the number of seropositive calves between the ages of 8-10 months was analyzed, they were shown to carry BRSV, PI-3, BAV-3 and BCoV infections (Table 4). This outcome is in agreement with the results of Hägglund et al. (2006). In many studies (Stott et al., 1980; Kimman et al., 1988; Van Der Poel et al., 1993), it has been shown that calves most likely become re-infected by BRSV and PI-3 at 9 months of age. In addition, the increasing in the number of calves seropositive for all infections at 8 months during the sampling period indicates that the studied viral agents were simultaneously circulating under natural conditions (Tables 4 and 6).

Despite other agents, there was an increase detected in the number of seropositive calves and in the geometric mean antibody titer for BVDV in the second month of age (Tables 4 and 5). The antibody response against BVDV can reach a detectable level at 2–3 weeks after infection (Howard et al., 1992). In addition to maternal antibodies, early-life infections may also cause an increase in this antibody. Thus, it can be postulated that the situation observed indicates that the BVD virus may circulate in the early period of life after birth. Another possibility is an intrauterine infection just prior to parturition. Hence, some calves can be seropositive at month 1 due to primary BVDV infection rather than maternal immunity. For PI-3, an increase in the number of seropositive calves at second month occurred, but unlike BVDV, the geometric mean antibody titer value was decreased (Tables 4 and 5). The development of a low titer antibody response in infected calves and high rates of maternal antibody catabolization are thought to be the causes of the reduction in the geometric mean antibody titer for PI-3. Here, the presence of early-period infection can also be postulated.

A high BRSV mean antibody titer was observed during all sampling periods (Table 5). Antibody titers against PI-3, BVDV and BCoV reached the lowest level at the 4th month; for BAV-3, they reached their lowest level at the 6th month. The amount of maternal antibodies varies depending on the dam's immune status as well as colostrum quantity and quality. The total degradation of

Table 6
Number of calves seroconverted by months.

	Sampling periods (months of age)[a]						
	2nd m.	3rd m.	4th m.	6th m.	8th m.	10th m.	12th m.
BRSV	0	2	4	0	14	6	2
PI-3	8	1	2	19	30	14	5
BHV-1	3	8	6	4	26	3	14
BVDV	16	0	8	20	17	16	1
BAV-3	3	9	5	28	31	19	13
BCoV	1	1	0	8	9	8	1

[a] Due to having the preliminary data's from the calves there was no seroconversion represented in the first month.

Table 7
Antibody titers of sampled dams and their offspring's in their 1st month (n: 94).

	Antibody prevalence in dam's (%)	Antibody Titer's	
		Dam's	Calves (1st m.)
BRSV	100	323.3	536.9
PI-3	93.2	75.7	54.9
BHV-1	35.9	2.5	1.9
BVDV	96.6	104.2	86.5
BAV-3	96.6	58.2	61.9
BCoV	93.2	243.1	172.4

maternal antibodies against BRSV, PI-3, BHV-1 and BVDV is reported to require more than 6 months (Baker et al., 1986; Van Der Poel et al., 1999; Fulton et al., 2004).

The numbers of seropositive calves against BHV-1 and geometric mean values of antibody titers were quite low compared to the other studied viruses, including in the first month of sampling. Therefore, conclusion on the maternal antibodies against BHV-1 will not be adequate. It is considered that the sampled calves may have low antibody titers against BHV-1, as shown by the limited number of seropositive calves.

As observed in the data, the number of seropositive calves started to decline in the second month for BRSV, BHV-1 and BAV-3, whereas it started decrease in the third month for PI-3, BVDV and BCoV. The antibody titer was at the lowest level in the 2nd month for BRSV, in the 4th month for PI-3, BVDV, and BCoV and in the 6th month for BAV-3; very low levels in every sampling period were observed for BHV-1. Calves with new infections between months 4 and 10 were found for all the studied viruses (Tables 4 and 5). Based on these data, the first vaccination time should be between the 2nd and 4th months of age. To minimize the negative impact of the vaccine on passive immunity and to provide a stronger immunity, a booster dose is also recommended one month after the first application. Re-circulation of all the studied viruses in parallel with an increase in the number of seropositive calves was observed between months 8 and 12 (Table 4), indicating that a third vaccination can be useful at this period.

In contrast to previous reports (Norström et al., 2000; Yeşilbağ and Güngör, 2008), according to the changes in number of seropositive calves, no difference was found among the large-, medium- and small-scale enterprises for the prevalence of BRSV, BCoV and BAV-3 infection. Thus, the importance of herd size may be matter of discussion for these viruses. In earlier studies (Yeşilbağ and Güngör, 2008; Raaperi et al., 2012), studied populations were sampled only once, with the relationship between herd size and infection seroprevalence being determined. In the present study, the sampled population was followed up for 1 year (8 times). Consequently, the difference between this study and earlier ones may originate from the different survey methods. At this point, the number of seronegative individuals in the herd and the prevalence of infection can be more relatable to the prevalence of infection than the herd size. Some other factors, such as the route of transmission, propagation velocity, farm-animal density in the region, direct contact rate of the animals, number of visitors, and biosafety rules, should also be taken into consideration. Given these criteria, when the capacity of a herd is increased, direct contact between animals and the number of visitors will increase automatically. Therefore, herd size can be classified as one of the main risk factors for respiratory infections but not the only reason.

When the transition of maternal antibodies was examined for correlations between the antibody titer in dams (n: 94) and their offspring in their 1st month (n: 94) for BRSV, PI-3, BHV-1, BVDV and BAV-3, a significant positive correlation was found, as expected. Despite high values of BCoV mean antibody titer, no correlation was detected (data not shown). One of the possible causes of this situation is the implementation of vaccine against BCoV at the end of pregnancy to increase the antibody titer in colostrum. In this case, the antibody titer in the dam rapidly declines after birth, while maternal antibody catabolization can occur at a normal pace in calves.

In overall there was an increase in the number of seropositive calves for all the viruses after 8th month of the sampling period. Similar to previous reports (Stott et al., 1980; Van Der Poel et al., 1993), these periods correspond to the winter season. However, in this study, 80 animals showing clinical symptoms were distributed as follows: 1.25% in spring, 27.50% in summer, 36.25% in autumn and 35% in winter (Table 3). Thus, the obtained data confirm that seasonal changes can be one of the most important risk factors for respiratory viral infections ($p < 0.001$).

5. Conclusion

Maternal antibodies received from colostrum start to decline from the 2nd month. Calves mostly encounter a viral infection for the first time between 4 and 8 months of age, though BVDV and PI-3 infections may occur in the very early stages of life (the first month). According to results of this study, a first vaccination of calves against respiratory viruses between the 2nd and 4th months is recommended. Due to the nature of inactivated vaccines, a booster dose is also advisable. Another important result of this study is the possible introduction of infections after the 8th month of age. Therefore, re-vaccination of animals at 6 months after the booster dose may be useful. To extend the level and duration of maternal immunity, vaccination of dams may be beneficial prior to the birth of the calf. The elimination of animals latently infected with BHV-1 or persistently infected with BVDV should support the health status of herds.

Conflict of interest

The authors declare that there are no conflicts of interest.

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